SOME BIOLOGICAL AND CHEMICAL ACTIVITIES OF ALBERTA SOILS.

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April 14, 1928.

U N I V E R S I T Y O F A L B E R T A .

FACULTY OF AGRICULTURE.

The undersigned hereby certify that they have read and recommend to the Committee on Graduate Studies for acceptance, a thesis entitled "Some Biological and Chemical Activities of Alberta Soils", submitted by Robert Henry Bedford, B.Sc., in partial fulfilment of the requirements for the degree of Master of Science.

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A THESIS

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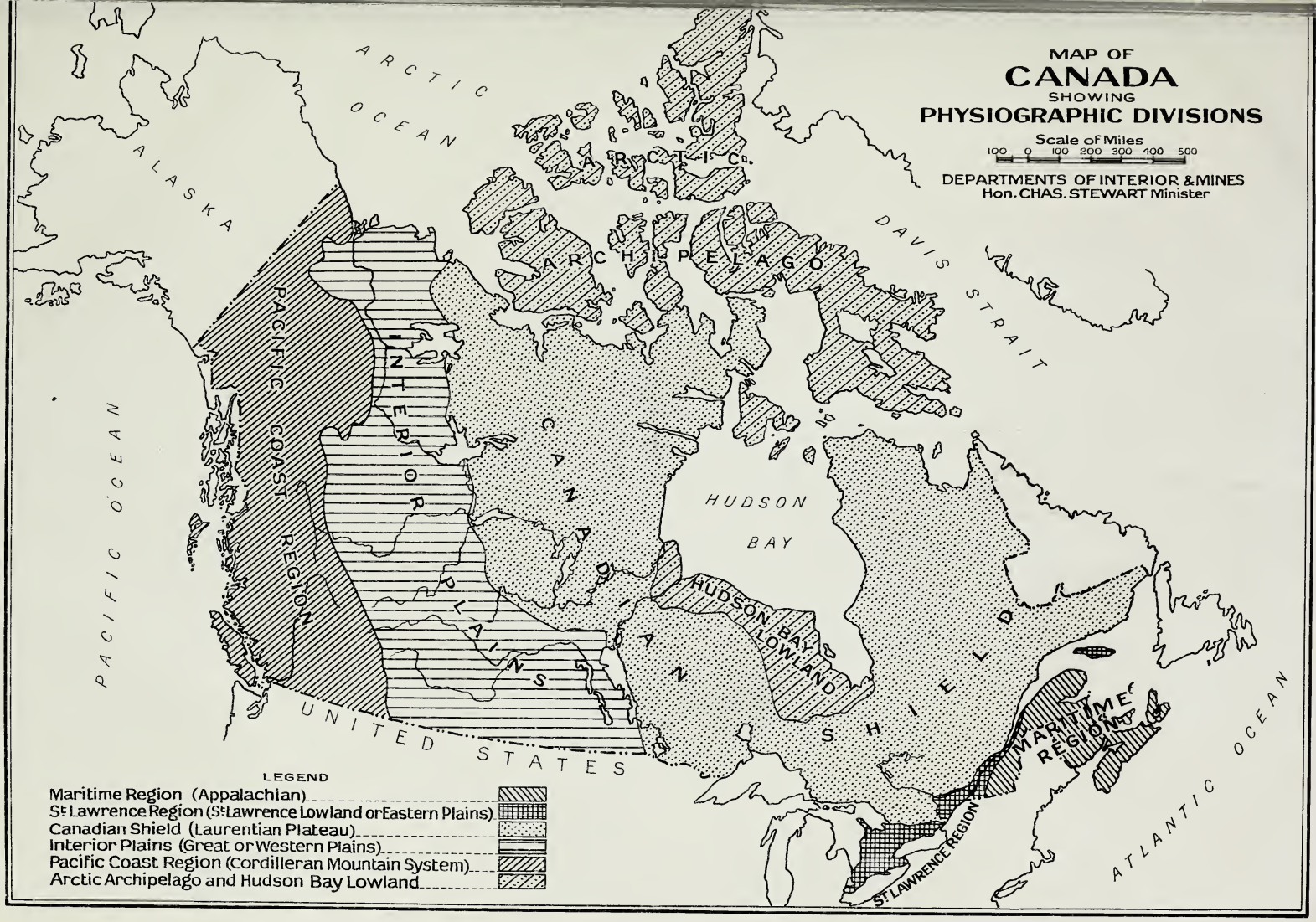

Physiographic Divisions of Canada.

Alberta occupies a portion of the Interior Plains.

SOME BIOLOGICAL AND CHEMICAL ACTIVITIES OF ALBERTA SOILS.

by

Robt. H. Bedford,

Department of Soils,
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INTRODUCTION.

Soil conditions and plant growth are the resultant of climatic factors and geological development.

If that portion of the Great Central Plain which is occupied by the Province of Alberta is considered in relation to its westerly boundary and northern topographical features, it may be supposed that both of those fundamental conditions above mentioned would have varied in respect to different sections of the Province over such a period of time to cause what is now designated as different soil groups, which are divergent amongst themselves in respect to the position of certain strata of minerals, as calcium carbonate, in relation to their proximity to the surface, the amount of vegetation that has become incorporated with the disintegrated rock and so forth. But in spite of these very apparent divergencies certain of these soils, as for instance, Prairie, Parkland, and Timber Groups, retain the inherent ability to produce

relatively high yields of agricultural crops under certain climatic conditions; and it is an endeavour to measure some of the soil factors that are known to be essential for plant growth, correlate them if possible with some apparent common property which appears to be an inherent quality of fertile soils, that forms the nucleus of this dissertation.

Four main divisions of soils are dealt with, namely, Prairie, Parkland, Timber, and Peat; and the factors measured are nitrogen derived from ammonia and nearly related compounds, nitrate nitrogen, as well as certain groups of microorganisms. The sample of timber soil used is representative of the best of its kind in the Province, and consequently shows a rather high total nitrogen content. The peat is essentially raw sphagnum; and the other samples are typical of their groups.

HISTORICAL.

Ammonification and nitrification are fascinating themes of interest to the microbiologist. The production of ammonia in soils was demonstrated to be a biological phenomenon by Muntz and Coudon in 1893; and that nitrification was due to a similar cause was first indicated by Pasteur thirty years previously; but it remained for Schloesing and Muntz in 1877 to offer tangible proof of the biological nature of the process.

The significance of these processes is that the higher plants, requiring nitrogen for their metabolism, assimilate it in the form of nitrates.

The questions then which arise are:

1. Whether microorganic activity can be correlated with either ammonification or nitrification;
2. Do the methods for differentiating these factors portray a true picture of those conditions;
3. Can soil fertility be measured by nitrification?

There is no unanimity of opinion amongst investigators regarding questions 1 and 2, but No. 3 is suggested by Waksman as being indicative of crop-producing power.

Various methods have been used to measure these factors. Remy (41) in 1902 suggested the use of solutions for the quantitative estimation of different physiological reactions, such as ammonifying and nitrifying power of different soils. This has been criticised because certain organisms are, nitrifiers for instance, are more sensitive to organic substances in solution than in the soil; but on the other hand microorganisms from sewage (organic matter) act much more energetically in liquid media.

Stevens and Withers (60) found no correlation between microbial activity of the soil and soil suspensions, a condition confirmed by many other investigators (65) and opposed by an equal number. Lohnis (65), for instance, found that Remy's method was quite as efficient in nitrification studies as when the natural soil was used, wherein definite quantities of a substance were added to known weights of soil and incubated for a specific length of time. Brown (12) found that there was a direct correlation between the ammonifying power of soils determined by Remy's liquid medium method and crop production.

Russell (51) states that Arnd found that in acid soils, such as peat, lack of nitrification did not necessarily indicate the absence of nitrifying organisms; and although the application of lime increased nitrification by creating a neutral reaction, a large excess might produce injurious effects which he attributed to microorganic activity in denitrifying the nitrates.

The concentrations of added material have markedly different effects on the formation of nitrates (31). Burgess (14) using the soil method found that nitrification was indicative of soil fertility; Prince (46) reported definite correlation between crop yield, numbers of microorganisms, and nitrifying power of certain plots studied at New Jersey. When highly nitrogenous organic matter, such as dried blood, was added to the soil its effect on nitrification was reflected by the soil reaction, alkaline soils inhibited the formation of ammonia, and acid soils were benefited.

Waksman (69) concludes that a study of numbers of microorganisms in the soil and of nitrification yield information for the differentiation of soil fertility.

A summary of the literature on the two phases, ammonification and nitrification, points to the conclusion that the former cannot be used as an indication of fertility, since there is no method extant which can definitely isolate ammonium compounds as differentiated from such closely allied substances as amides and imides; but on the other hand the general concensus of opinion is that nitrification, together with numbers of microorganisms, offers

a basis for the classification of soils.

The measurement of carbon dioxide evolved from the soil is of more recent origin, although as early as 1880 Wollny found that the carbon dioxide content of the soil varied with the organic matter present. Gainey and Neller (25) found a correlation between ammonia and carbon dioxide, and Neller (43), using pure bacterial cultures, found a high concentration of carbon dioxide corresponded with a high ammonia content; while Waksman (71) suggests the measurement of carbon dioxide as a method for grading soils on the basis of their fertility. Russell and Appleyard (53) investigated the decomposition of soil organic matter on different plots at Rothamsted and found a definite correlation between carbon dioxide, nitrate nitrogen, and bacterial numbers; a fundamental interrelationship in soil bacteriology.

THE PROBLEM.

The differences in the chemical and physical nature of the four main soil groups of Alberta, and their ability to produce correspondingly high yields of agricultural crops under optimum climatic conditions, suggest that there must be some inherent factors common to each soil group which plays an important part in the support of vegetative growth. Plant growth reflects to a marked extent the results of microorganic activity, which in turn may be correlated with specific chemical substances found in the soil as a result of biochemical decomposition of the organic matter. Instance, for example, the production of ammonium compounds, nitrate nitrogen, and carbon dioxide. Measurements then

have been made of two factors:

1. Chemical: ammonium and closely related compounds, nitrate nitrogen; and, in the case of peat, of carbon dioxide.
2. Biological: total numbers of microorganisms, and, in the case of one soil (Parkland), of two other groups, namely, fungi and actinomyces.

and a comparison made of the response of the different virgin soils under specific treatment.

EXPERIMENTAL METHODS.

The virgin soils used in this investigation were obtained from the following locations:

a. Prairie - Hand Hills, Hanna.
b. Parkland - Edmonton.
c. Timber - Pigeon Lake.
d. Peat - Edmonton.

They were air-dried and with the exception of the peat, sifted through a coarse-mesh sieve.

This outline of experimental methods is divided into three sections: (1) ammonification and nitrification; (2) carbon dioxide evolution; (3) miscellaneous. And each of these sections subdivided into: (a) chemical and physical, and (b) biological divisions:

Section (1) - Ammonification and Nitrification.

(a) Chemical and Physical.

The hydrogen ion concentration of each air-dried soil was established by the colorimetric method using Clark and Lub's system of indicators.

Ten grams of soil and 50 cc. of water were placed in a shaker bottle, rotated for ten minutes, filtered, indicator added, and compared against a standard in comparator block.

Determination of optimum moisture. A measured quantity of water was allowed to percolate through 100 grams of soil and the gravitational water drained through was measured. Sixty per cent. of the water-holding capacity was taken as the optimum moisture of the respective soils.

Quantities of soils and treatments. One hundred grams of soil were placed in small jelly jars in triplicate, and subjected to the following treatments and rates of application:

Control	optimum moisture	60% capacity.
Phosphorous	mono-calcium acid phosphate.	0.1 grams.
Calcium	calcium carbonate	.5 "
Calcium and blood.		2.0 "
Blood alone		2.0 "
Ammonia	ammonium sulphate (2 cc. - 10% soln.)	.042 " nitrogen.

Optimum moisture was added (an additional 2 cc. for each gram of dried blood), and the soil incubated at room temperature for definite periods of 1, 2, 3, 5, 8 and 11 weeks. At the end of the incubation periods the soils were dried at 80°C. for sixteen

hours excepting sufficient soil for microbial counts. The dried soils were then ground to pass an 80-mesh sieve, stored in sealers and later analysed for ammonia and nitrate nitrogen, as follows:

Ammonia: determined by aeration in the cold. A definite quantity of soil and salt mixture consisting of sodium chloride and sodium carbonate, are placed in a long glass tube which is connected to an absorbing tower containing a definite amount of standard acid; the whole is then aerated for three hours by means of a suction pump, and the acid titrated against standard alkali, using methyl red as an indicator (42). In the case of peat soils this method had to be slightly modified on account of the bulky nature and difficulty of wetting of the ogranic matter. Five grams only of peat could be used in the tubes as opposed to fifty grams of mineral soil, to which was added one hundred cc. of salt mixture and the whole allowed to stand until the peat was completely saturated. A few glass beads placed at the bottom of the aeration tube permitted a more easy ingress of air.

Determination of nitrate nitrogen. This method consists of leaching out the nitrates from the soil and determining their presence colormetrically by means of phenol-disulphonic acid. The presence of chlorides from the addition of blood necessitated the addition of silver sulphate solution. Slight modifications of the method were found necessary in determining nitrates in peat: (27)

1. Peat is difficult to wet: shake for 30 minutes and filter instead of decanting the supernatant liquid;

2. The colour of the extract is more intense than in mineral soils: increase the carbon black from 1% to 2%;

3. In each instance where agitation is a necessary part of the regular procedure, it was found that by increasing the time 50%, and combined with the above modifications, a clear solution was ultimately obtained.

(b) Biological.

In the case of the control in each soil group plate counts were made of (1) bacteria, (2) fungi, and (3) actinomyces; in the other treatments only bacterial counts were made. The whole of the biological work was done in the evenings so as to obviate unnecessary disturbance of atmospheric conditions in the laboratory. The following media were used:

1. Bacterial counts

Beef extract-peptone-agar adjusted to + 1% acidity.(22)

2. Fungi:

This medium having a pH 4.0 was proposed by Waksman and gave very satisfactory results (68).

3. Actinomyces:

Another of Waksman's media was used in this case and gave satisfactory results. This medium had a pH of 5.7 (67).

These methods of differentiating between the groups of soil microorganisms are designated under the term "Physiological selection".

Counts at the end of six days are recorded in this thesis. (Table 15).

Section (2) - Carbon Dioxide Evolution of Peat.

Chemical and Biological.

All the necessary constituents for the respective treatments, excepting the peat, were first placed in a 500 cc. Erlenmeyer flask and then 25 grams of finely-ground well-mixed peat added. Where calcium carbonate was used the precaution was taken of leaving it in contact with the solution for a period of time before adding the peat so as to obviate the danger of measuring the carbon dioxide evolved resulting from the reaction between the added chemical substances. Glass wool was inserted between the solution and the peat; this necessitated the capillary movement of moisture and dissolved substances and at the same time permitted an even distribution over the peat of the microorganisms contained in the inoculum, as well as permitting them to live in a more stable environment as the solution percolated throughout the solid media. To the flask was fitted a rubber stopper through two holes in which glass tubes were inserted, one reaching to the bottom of the flask permitting the ingress of air, and the other just reaching below the stopper for the egress of carbon dioxide; a small plug of cotton batting being inserted in this tube to stop any small pieces of organic matter from passing out with the carbon dioxide.

The incubation flask was then connected to an absorption tower containing glass beads and 50 cc. N.4 sodium hydroxide solution, which was changed and titrated each morning using standard hydrochloric acid and indicators phenolphthalein and methyl orange (64).

Eighteen litres each of standard hydrochloric acid and sodium hydroxide solution, sufficient for the duration of the experiment, were made up. The former was protected against the ingress of ammonia fumes in the atmosphere, and the alkali solution protected by means of two soda lime tubes, one attached to a rubber bulb used for pumping air into the container and the other attached to the end of the 50 cc. pipette used for measuring the alkaline absorbing solution, which was inserted through an aperture in the stopper of the demi-john.

In order to stop any back flow of carbon dioxide from the incubation chamber into the absorption tower next preceding it, a screw clamp was fixed on the rubber tubing connecting the absorption tower to the air ingress tube of the incubation chamber. This clamp was released immediately after the suction pump was started and the solution began to rise in the absorption tower. The whole system, connected in series, was subjected to fifteen minutes aeration each day, immediately prior to titration; but the carbon dioxide evolved had free access to wander into the alkaline absorbing medium at any time.

The atmosphere drawn through the system was first washed by passing it through washing towers containing a 30 per cent. potassium hydroxide solution, and at the other end of the system a similar tower was installed to wash any air passing back into the system when the suction was released.

Section (3) - Miscellaneous.

(a) Chemical.

Reducing sugars from straw. A mixture of 5 grams of Marquis wheat straw and 125 cc. of a definite concentration of phosphoric acid were placed in a 250 cc. Erlenmeyer flask, stoppered with cotton batting, allowed to stand overnight, and then subjected to steam pressure in the autoclave under the following conditions:

Sufficient heat was generated to raise the temperature in the autoclave to 120°C. in 15 minutes, retention of this temperature for 75 minutes, and then the autoclave was exhausted in 10 minutes (57). The straw mixture was immediately filtered through a No. 40 Whatman filter and the filtrate analysed by the Bertrand method for reducing sugars (4).

Total nitrogen was determined by Gunning's modification of the Kjeldahl method (4).

The determinations of carbon in peat were made by means of the "Liebig" combustion apparatus.

In all instances where numerous similar analyses necessitated the constant use of graduated apparatus, the same measuring instruments were used throughout the particular analysis. This safeguard is extremely necessary in such analyses as those of nitrate and sugar determinations, where so much mechanical manipulation forms a part of the methods.

(b) Biological.

Fixation of atmospheric nitrogen. A definite quantity (70 cc.) of the straw filtrate was placed in an 800 cc. Kjeldahl

flask, inoculated with 10 cc. of inoculum from previously isolated free nitrogen fixing organisms, and incubated for a definite time under laboratory temperatures.

EXPERIMENTAL RESULTS.

Before entering upon a discussion of the results of the problem under investigation, it is desired to draw attention to certain factors, fundamental ones in the writer's opinion, of soil constitution, which should not be treated in the customary abstract manner but rather oriented to a position that is in juxtaposition to the results which reflect their potentialities. Take, for instance, the microorganic population. The customary procedure is to compare this factor in the different soils irrespective of the source from which they obtain their energy. If it is conceded that the physiological activity of micro-organisms is a kinetic reflection of the source of potential energy - the organic matter - conditioned by the soil solution; that is to say, for example, the hydrogen ion concentration may be such as to favour the growth of a dominant flora such as fungi, in which case physiological activity would be reflected in a small accumulation of nitrate nitrogen, then the question naturally arises whether the comparison of biological activity (numbers and nitrates) of two or more different soils would be justified if their organic matter content were widely divergent, and no cognizance taken of this fact.

In the writer's opinion they would not be comparable because:

1. The organic matter is the source of energy for micro-organisms.
2. The organic matter is the source of nitrate nitrogen.
3. The fertility of our semi-arid soil (Prairie) cannot be explained owing to the slight increase in bacterial numbers when based upon the total soil aggregate.
4. Plant growth is indicative of the rate of availability of essential elements, and no comparison is possible between soils containing wholly different quantities of organic matter, unless cognizance is taken of this fact.

On the other hand, there should be a relationship between the numbers of bacteria per gram of organic matter, nitrate nitrogen per gram of organic matter, and the total organic matter for each 100 grams of soil; and in the writer's opinion there is.

Salisbury (54) in discussing the stratification of Woodland soils observes that the vertical distribution of microorganisms may be directly correlated with the distribution of organic matter, each varying inversely with the depth.

The ensuing discussion will consider the effect of:

(a) optimum moisture
(b) phosphorus. (mono-calcium acid phosphate)
(c) Calcium carbonate
(d) calcium carbonate and blood.
(e) blood
(f) Ammonium sulphate

upon the decomposition of the soil organic matter, reflected by measuring the three factors ammonification, nitrification, and microbial numbers. The question arises what are the relative quantities of organic matter and nitrogen present, and the hydrogen ion concentration in the respective soil groups.

Table 1. - Total nitrogen, organic matter, and pH in the four soil groups.

Soil Group.	Organic matter per 100 g. soil.	Nitrogen per 100 g. soil.	Nitrogen per 100 g. org. mtr.	Ratio N/O.M.	pH
	%	%	%		%
Prairie	7,18	.240	3.34	1:29.9	6.6
Park Belt	27.08	.918	3.39	1:29.5	6.8
Timber	22.22	.609	2.74	1:36.5	6.9
Peat	93.13	.800	.87	1:116.4	3.5

Determination of organic matter by ignition.

The Effect of Moisture on Microorganic Activity.

The addition of water to a soil brings into solution its characteristic elements or compounds till a definite concentration is reached; and, seeing that bacteria are living organisms requiring certain compounds in solution for their metabolism which are contained in their edaphic environment, it is only natural to expect that they should respond to this

enhanced availability of nutrients by increased numbers. This they do but only to a certain point, for their numbers are subject to fluctuations characteristic of living matter dependent upon a constantly changing environment. Reference to Table 2 will show the response of the microorganisms to a changing environment, as well as a rhythmic fluctuation in the case of the Park and Timber groups, and although this does not appear in the Prairie soil, but rather a steady increase, the fact must not be overlooked that this rise coincides to a similar condition for those particular periods of incubation of the other two mineral soil groups. This is rather significant and points to the necessity of making frequent counts. A similar fluctuation exists in the Fungi group, but the Actinomyces show a steady rise throughout the period under observation. They are slow growing (67) and their physiological activity would be reflected in numbers, a probable measurement of the time factor required for completion of their life cycle, i.e., cell division.

Table 2. - Microorganisms, millions per gram of soil in the four soil groups.

Incubation in weeks.	Microorganic group.	Prairie*	Park Belt	Timber	Peat
	Control	.10	2.16	1.37	.03
1	Bacteria	----	3.34	4.89	1.26
	Actinomyces	----	----	----	----
	Fungi	----	----	----	----
2	Bacteria	2.60	1.60	1.65	1.60
	Actinomyces	----	----	----	----
	Fungi	----	----	----	----
3	Bacteria	----	1.60	1.61	1.57
	Actinomyces	----	.29	.28	.09
	Fungi	----	.14	.11	.19
5	Bacteria	5.20	3.22	4.95	1.57
	Actinomyces	.46	.58	.69	too dense to count.
	Fungi	.05	.07	.12	.15
8	Bacteria	----	1.57	1.61	3.12
	Actinomyces	----	.15	.14	.13
	Fungi	----	.28	.39	.14
11	Bacteria	7.68	12.10	3.28	1.67
	Actinomyces	.92	.46	.52	.13
	Fungi	.11	.33	.50	.37

* Only three incubation periods were possible owing to insufficient soil.

The microorganisms of peat cultured on media similar to that utilised in the differentiation of groups in the mineral soils are characteristic of one group only - the Fungi. There would appear to be no doubt that they dominate the biological activity therein, which observation we shall see later is substantiated by a measure of nitrification. This not only suggests a wholly different microflora and fauna are present but indicates a biological adaptation of one group to a wide range of hydrogen ion concentration, for the three media used corresponded to a neutral medium for the isolation of bacteria, a pH 5.75 for Actinomyces, and pH 3.9 for Fungi.

Observation of Table 2 indicates that there is a fairly close analogy in the numbers of microorganisms within the four soil groups. It is particularly noticeable that peat, a very unfertile environment for normal plant growth stands in a direct relationship with the other soils, a condition that, in the writer's opinion, is not justified by experimental evidence but is the reflection of a method used that is not sound in principle for a basis of comparison and which has become orthodox for all comparative studies irrespective of the factors under consideration merely as a result of repetition. If it is willingly conceded that microorganisms obtain their energy from the soil's organic matter, then it is only logical to suppose that their numbers should be based upon that factor, and on the assumption that the respective volumes of the mineral soils are practically similar, the quantity of organic matter in 1 gram of soil should form the basis of calculation. It will later be shown (p.25)

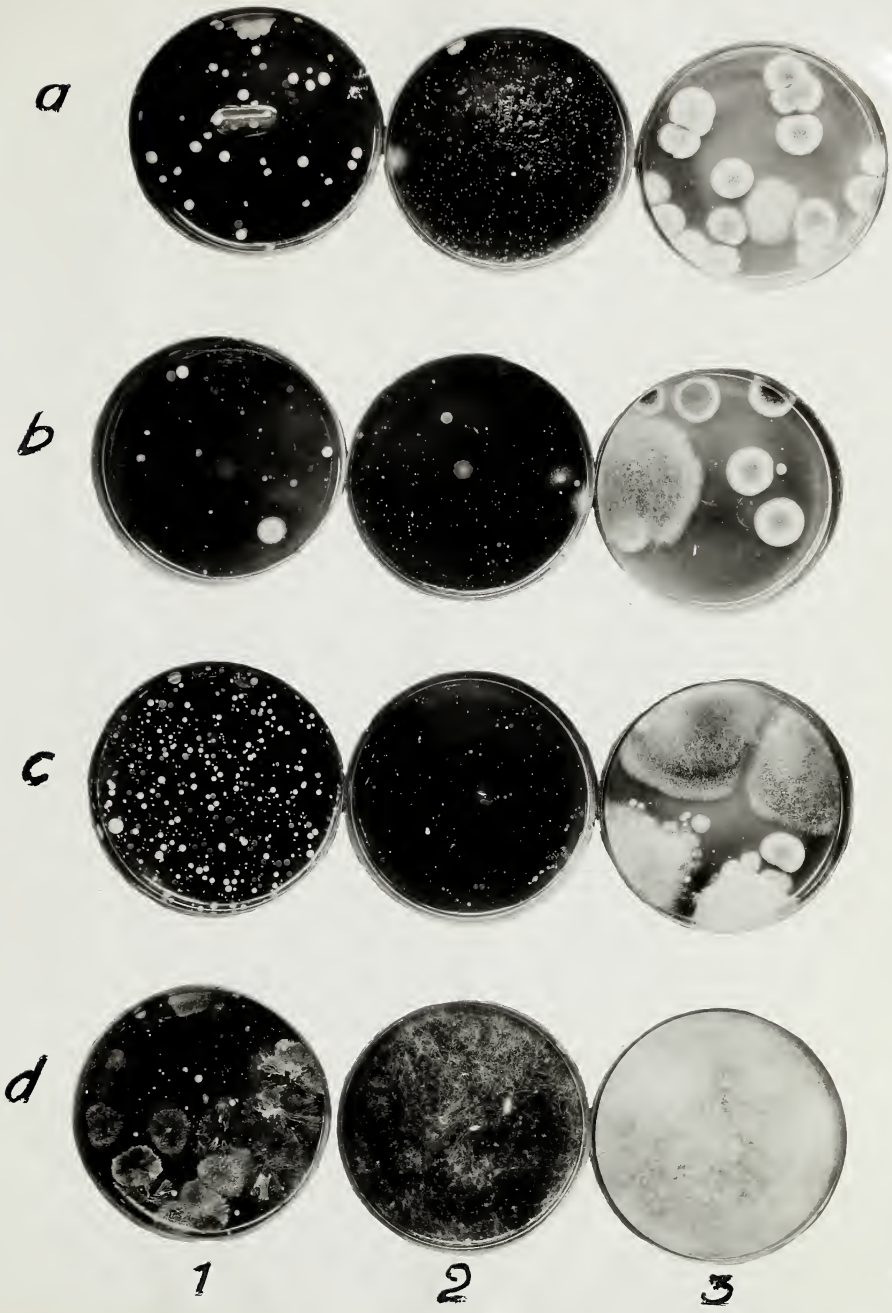


Plate I. Comparison of microorganisms of four soil groups.

(a) Prairie
(b) Parkland.
(c) Timber.
(d) Peat: 1. Bacteria, 2. Actinomyces, 3. Fungi.

that rational correlation may be made on this basis. A graphical representation on both bases is shown in Figure 1, wherein it will be observed the new method of computation places the number of microorganisms of peat in a direct relationship to its condition of fertility.

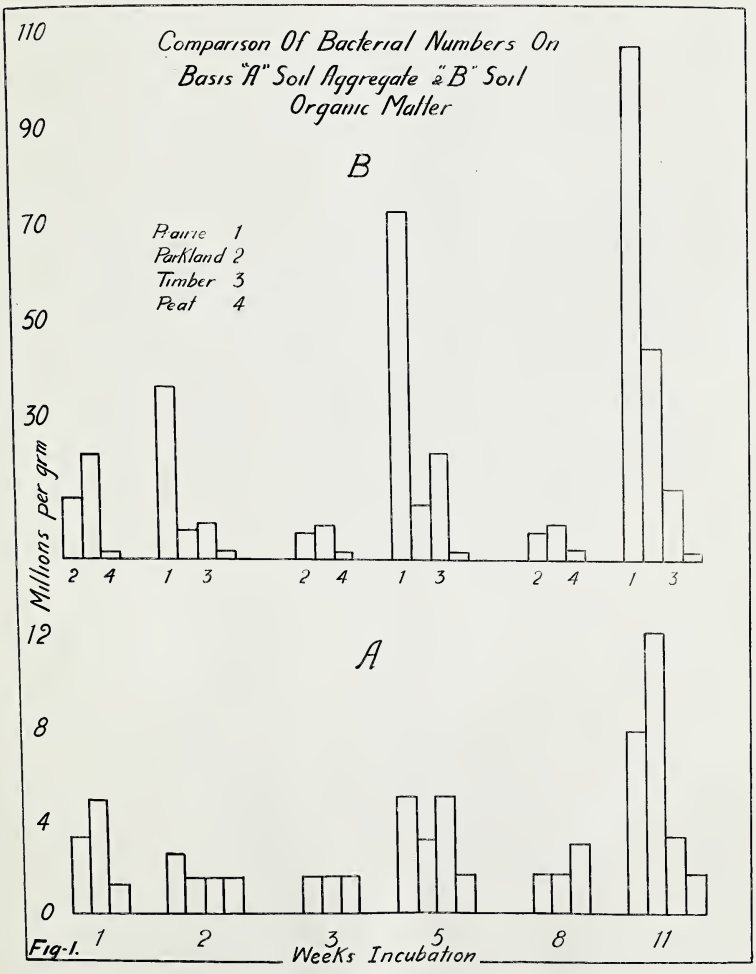


Figure 1.

The question then with which we are confronted just now is that of fluctuating numbers. Is that phenomenon peculiar to artificial laboratory conditions, or is it an inherent quality reflecting an organized subterranean population of biological forms? Russell (51) shows rather conclusively that a living organism is responsible for this rhythmic sequence. Evidence recently accumulated (16) lends weighty support to the phenomenon of biological organization, for Rothamsted investigators found that the numbers of bacteria were definitely inverse to those of protozoa, that this relationship was independent of moisture and temperature, and that these fluctuations were daily as well as seasonal. It has been shown that protozoa are practically universally present in soils; their presence has been qualitatively demonstrated in the soils under investigation.

These fluctuating numbers of microorganisms are not then a responsive factor of artificial laboratory conditions, but an inherent function of biological activity. The significance of this is obvious, and we shall have recourse to this biological phase a little later (p.24).

There are certain groups of organisms present in the soil that obtain their energy by oxidation of simple inorganic nitrogen compounds. Russell (50) maintains that very small quantities of ammonia or nitrites are ever found in the soil. He infers, therefore, that nitrate formation is the speediest of the three reactions and ammonia formation the slowest, which ipso facto, commands the process. It might be of interest then to follow the course of nitrate formation throughout the experi-

mental period and later to see whether there is any common ground between the biological factor, the genesis and finale of the nitrate radical.

The Effect of Moisture on Nitrification.

It has been observed that the effect of the addition of moisture to the soil was to increase the biological activity therein, and seeing that this activity is reflected in increased numbers there should obviously be an increase in metabolism which should be signified by an accumulation of nitrate nitrogen. Reference to Table 3 indicates that there was a steady increase in the formation of nitrate nitrogen in the three mineral soils, but the case of peat was anomalous.

Table 3. - Nitric nitrogen parts per million of soil.

Period of incubation weeks.	Prairie	Park Belt	Timber	Peat.
	Control 2.26	2.66	3.54	17.60
1	----	10.87	15.62	82.14
2	14.70	20.27	58.82	69.44
3	----	27.77	58.82	24.27
5	30.00	35.71	83.33	24.76
8	----	57.69	133.33	22.92
11	41.66	66.18	166.66	22.72

The writer offers the suggestion that this fluctuation in the case of the peat may be accounted for by the desiccation, in the process of air drying previous to the commencement of the experiment, of the vegetative fungal inhabitants, the dominant flora, at an earlier period than the nitrifying organisms, nitrosomonas and nitrobacter, which would result, in the initial stages, of an accumulation of ammoniacal compounds, because the acid reaction of the peat practically inhibits nitrification, but the increased formation of the base-forming compounds would tend to decrease the hydrogen ion concentration, a condition contributing to enhanced activity of the nitrifying organisms and a consequent accumulation of nitrates. The addition of water gradually reversed the process by causing germination of fungous spores and consequent assimilation of simple nitrogen compounds resulting in the fluctuations shown.

Inspection of Table 3 (p.22), wherein the computation of nitrates is upon the basis of the soil as a whole, would lead one to suppose that a certain obvious order of the rate of nitrification existed within the soil groups; but in the writer's opinion this position cannot be supported by the related factors - biological activity and organic matter present.

The Interrelationship of Factors, Organic Matter, Bacterial Numbers and Nitrate Nitrogen.

We have seen that coincident with an increase in the microorganic population from that at the beginning of the experiment there was an accumulation in nitrate nitrogen, and

seeing that nitrogen is derived from the organic matter of the soil, it naturally follows that these three factors suggest a basis of correlation; but the first question with which we are confronted is whether there is any significant relationship between the three factors soil aggregate, bacterial numbers, and nitrates. This there cannot be, because no differentiation can be made in regard to the different soils and they must therefore be represented as a linear curve from which condition it is obvious there cannot be any correlation with specifically varying factors, such as bacterial numbers and nitrates. But by resolving bacterial numbers and nitrates in terms of 1 gram of organic matter - a common unit - for each soil group a picture is presented which reflects the significant relationship. Observation of Figure 2A will clearly demonstrate this fact. The nitrate figures have been computed on the basis of the total quantity accumulated at the end of the experiment, divided by the number of weeks of incubation. It might be objected that on this basis a false picture is conveyed of the rate of nitrification. For when the numbers are actually low the accumulation of nitrates is similar to when the numbers are high, but although this objection has a degree of validity, it does not affect the principle involved, namely, that nitrates are a measure of bacterial numbers because the fluctuations in those numbers within each soil group is directly proportional to the fluctuations of nitrate nitrogen within each respective soil group. It is quite true that the ratio of nitrate formation for each group at the different periods of incubation must fluctuate and this must

have an accumulative effect, so that at the end of eleven weeks the differences will be more accentuated. This does not detract from the principle involved. For recourse to Figure 3A shows that the relative positions of the different soil groups is maintained throughout the experimental period. Again the numbers and nitrates are directly related. Reference to Figure 2B shows a similar correlation involving the total numbers of microorganisms and nitrate nitrogen accumulated and distributed equally over the six incubation periods.

It will be observed that there are apparently great divergencies from any parallelism in bacterial numbers, but as a matter of fact the picture is a true reflection of each soil group's organic matter content and microbial population, for there is an inverse relationship between numbers and microorganisms per gram of organic matter and total organic matter of 100 grams of soil, which formed the basis of this experiment, and which offers an explanation why Prairie soils so relatively low in organic matter are so fertile for a limited period compared to the other soil groups, because the rapid decomposition of the organic matter and consequent release of nitrate nitrogen and other salts, is merely a reflection of physiological activity conditioned by a greater degree of aeration owing to the particular physical structure of its inorganic framework. There are then these rather interesting facts, that the greater bacterial numbers in Prairie soil per gram of organic matter partially offset the apparent advantage of increased organic matter content of the Parkland and Timber soils, and that the

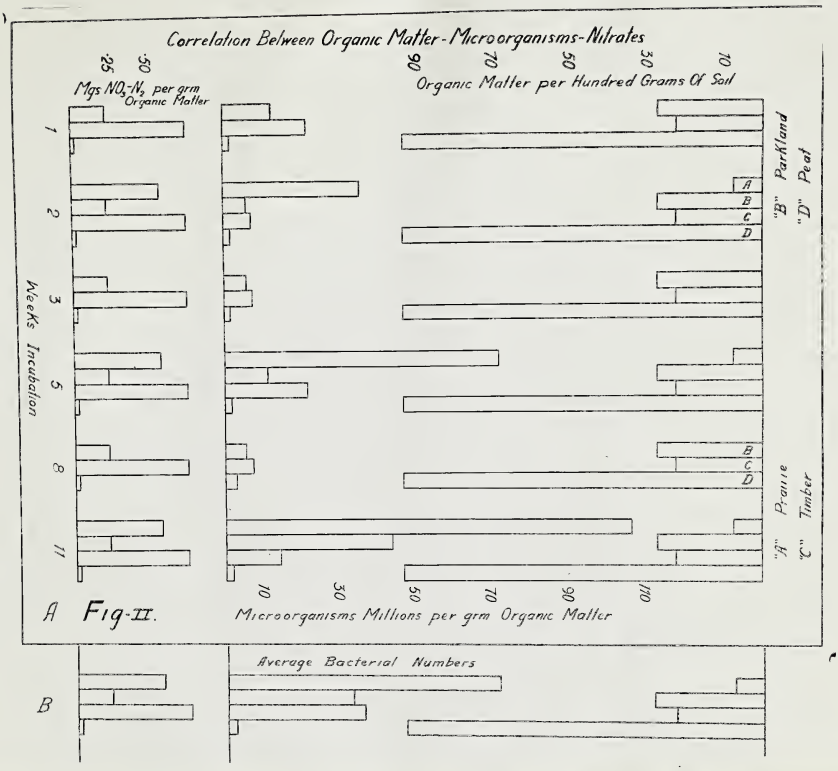

Correlation Belween Organic Matter-Microorganisms-Nitrates
Mgs NO3-N2 per grm Organic Matter
Organic Matter per Hundred Grams Of Soil
Weeks Incubation
"B" Parkland
"D" Peat
"A" Prairie
"C" Timber
Microorganisms Millions per grm Organic Matter
A Fig-II.
Average Bacterial Numbers
B

Figure 2 - A & B

condition of fluctuation in microbial numbers at the different periods of incubation are remarkably constant. If, on the other hand, the numbers of bacteria were based upon 1 gram of soil aggregate, the slight increase in numbers and the apparent low rate of nitrification are so opposed to the known fertility of prairie soils, that they suggest antagonistic factors rather than each directly reflecting the other. Hilgard (Marshall 41) attempts to explain the fertility of semi-arid soils on the basis of a narrower carbon/nitrogen ratio in consequence of which relatively greater quantities of nitrates are made available than in more humid regions. This view can be reconciled with experimental fact if the carbon/nitrogen is narrowed due to the relatively enormous numbers of microorganisms per gram of organic matter which displace the wider ratio existing in the vegetative organic matter by reason of their physiological activity, and they themselves supplying the source of nitrogenous material in the form of their cellular bodies, in which condition the proteins are much more easily decomposed.

It is a matter of common knowledge that, given optimum climatic conditions during the growing period, the crop yielded by the Prairie soil will be equal to that of any other mineral soil group, and the question naturally arises whether we can find any relationship between any of the factors measured so that the position of each soil group may be arranged in order of apparent productivity. Reference to Table 4 shows their relationship on the basis of the ratio of nitrate nitrogen to organic matter content of one hundred grams of soil, after eleven weeks incubation.

Table 4. - Relationship of the soils on basis of ratio of nitrate nitrogen to organic matter per 100 grams soil.

Soil Group	Mgs. NO_3-N_2 in 100 grm. soil.	Organic matter per cent. soil.	Mgs. NO_3-N_2 per grm. organic matter.	Ratio of organic matter to NO_3-N_2
Timber	16.7	22.22	.75	1,333
Prairie	4.2	7.18	.58	1,724
Park	6.6	27.08	.24	4,166
Peat	2.3	93.13	.025	40,000

When a comparison of these soil groups is based upon the accumulation of nitrate nitrogen per unit of organic matter within a given period of time they stand in the following order:

Table 5. - Order of comparison of soil groups under optimum conditions in relation to nitrification.

Soil Group	Comparative value on basis of 100.
Timber	100
Prairie	77
Park	32
Peat	03

It is not, of course, inferred that this position would pertain under extreme climatic conditions (i.e. deviation from the optimum within each group), for obviously the physical and chemical factors would exert a pronounced influence the more the climatic conditions deviated from both sides of the optimum - (Max. ← opt. → min.) - but that relationship will stand up to a certain point of climatic variability. This inverse relationship between the rate of nitrification and total amount of organic matter present is compensated for by the greater amount of organic matter present, and explains for instance why Park Belt soil with so low a comparative rate of nitrification is able to accumulate a larger quantity of nitrate nitrogen compared to say the Prairie soil, wherein the rate of nitrification is so much greater.

The serious objection might be raised that in choosing only one period - the last - for illustrating the point at issue, undue favouritism has been invoked to build a suitable foundation for a reason to depart from the orthodox system, but such is not the case, for examination of Figure 3B, wherein is delineated the comparative rate of nitrates accumulated per 100 grams of organic matter at the end of each incubation period for the four soil groups, will clearly demonstrate that the relative positions of the factors concerned are well maintained.

In view of the foregoing observation, it is the opinion of the writer that the disposition of the four virgin soil groups of Alberta in relation to their ability to form nitrate nitrogen is as graphically presented in Figure 3B, and not as in Figure 3A.

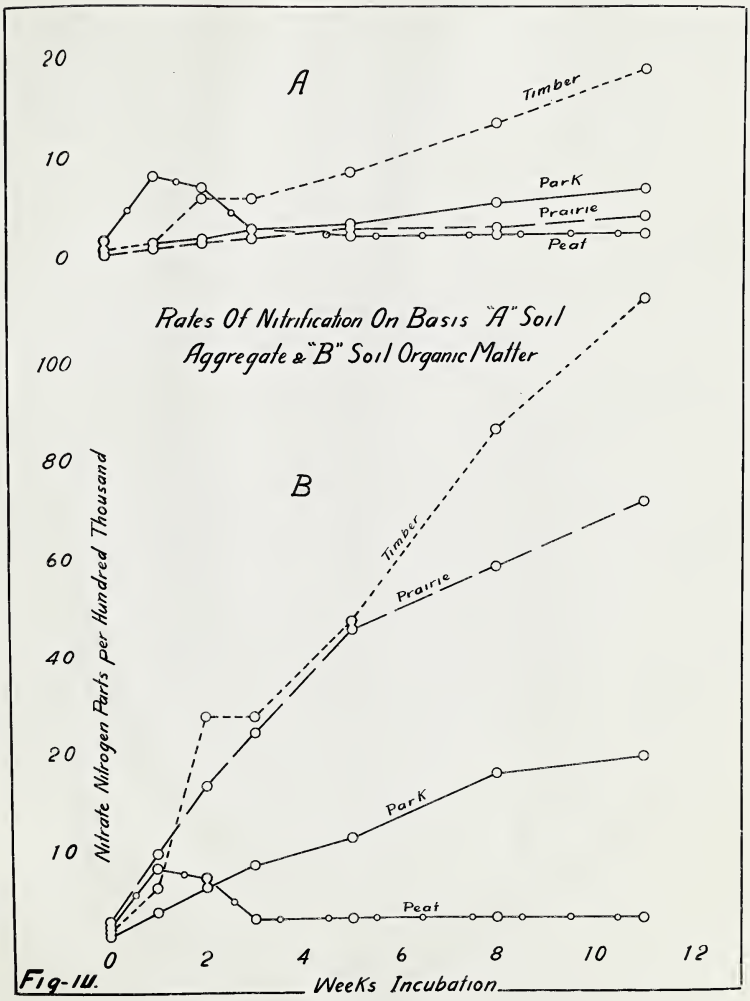
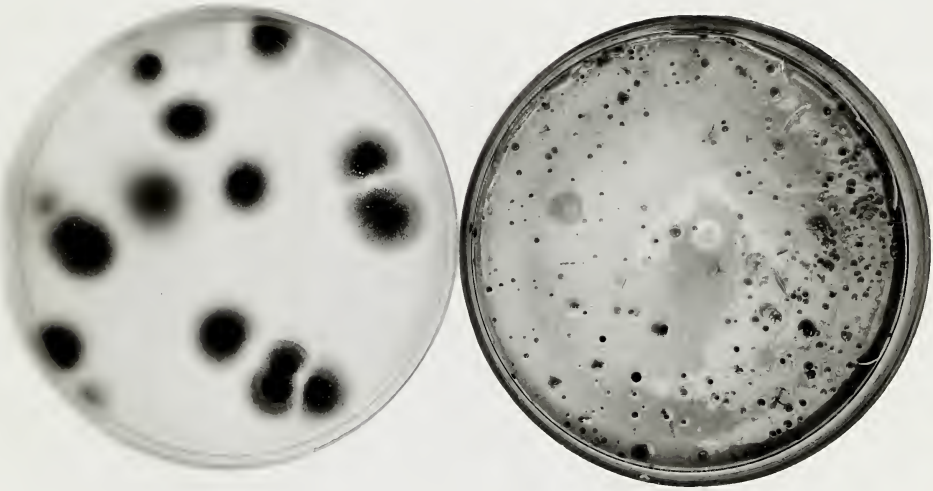

A
Timber
Park
Prairie
Peat
20
10
0
Rates Of Nitrification On Basis "A" Soil Aggregate & "B" Soil Organic Matter
B
100
80
60
40
20
10
Nitrate Nitrogen Parts per Hundred Thousand
Timber
Prairie
Park
Peat
0
2
4
6
8
10
12
Weeks Incubation
Fig-11.

Figure 3 - A & B

The Effect of the Addition of Fertilizers.

The addition of a chemical compound or element to a complex chemical system such as the soil solution may profoundly affect its original state, and if it is borne in mind that this system conditions the activity of a very animate population, which is obtaining its energy from the soil's organic matter, then any change in that environment may automatically be transmitted to the microbial population and ipso facto their products of metabolism.

A rather good illustration of the effect of changed environment occurred during last summer. The media which the writer had been using for isolating fungi had given characteristic results, Figure 4 A, and owing to the small quantity remaining in the flask not being sufficient for another whole series of plates, it was stored for about six months. Occasion then demanded its use, but instead of getting the characteristic growth there appeared colonies morphologically dissimilar, Figure 4 B. At the time of plating out the odour of the medium was foreign to the normal substance, and although it was kept thoroughly sterile, there had undoubtedly taken place a chemical change in the medium.



A B

Plate II.- A & B. Change of forms or groups of microorganisms due to change in chemical environment.

We are concerned with the effect upon biological activity and nitrification of the addition of the minerals mono-calcium acid phosphate, calcium carbonate, ammonium sulphate, and nitrogenous organic matter in the form of dried blood, to the different soils, and the question arises what has been the observed effect of these substances upon physiological activity of the microorganisms.

Phosphorus. The mechanism of the beneficial effect of phosphorus to living organisms is difficult to ascertain, but this much is known from experiment, that phosphates are essential to cell division.

Calcium. In the case of calcium, too little is known, but the suggestion is made (59) that calcium salts of proteins may be present in living cells but not as a constituent of the cell protoplasm. Apart from any direct effect there is the indirect one of soil reaction in which calcium salts act as efficient buffers in regulating the hydrogen ion concentration and so governing within certain limits the activity of micro-organic groups.

Blood. The addition of blodd provides a highly nitrogenous organic substance for microbial attack. In addition to the nitrogen compounds there are a number of other organic and inorganic substances (59) such as fats and sugars, (sources of energy), and sodium, potassium, calcium, etc., which base-forming elements tend to neutralise acids resulting from the rapid organic decomposition. Observation of Tables 9 and 10 (p. showing the results of nitrification when blood alone and blood

and calcium carbonate were added respectively to the soils point markedly to the ability of all the four soil groups to utilise easily decomposed nitrogenous material. The beneficial effect of the base-forming substance is apparent, in the greater accumulation of nitrate nitrogen where calcium carbonate has been added, with the exception of Prairie soil.

The increase in nitrate nitrogen in the case of peat is of particular interest because of the greater stimulation in the process of nitrification, and the fact of this abundance appearing in the sample where no calcium carbonate had been simultaneously added with the blood may be attributed to the neutralising effect of the base forming compound ammonia. This result at least establishes the fact that given a favourable environment the original grouping of the microorganisms is displaced to accord more nearly to a condition approaching that of a fertile soil, and in fact this observation is confirmed by a comparison of cultures grown from normal peat without any chemical treatment as a control, and that treated with blood, wherein there appeared bacterial colonies morphologically dissimilar.

It is, of course, appreciated that this increased formation of nitrate nitrogen may possibly have been derived from the blood in toto, but the point is, that given favourable conditions nitrification in peat is extremely active and that the original acid condition does not wholly preclude the presence of nitrifying organisms, which it has been previously observed are most physiologically active in a neutral or slightly alkaline medium.

Nitrification in Peat.

Effect of nitrogenous organic matter in peat, in eleven weeks.

Total nitrogen in 100 gr.peat.	NO_3-N_2 accumulated in 100 gr. peat.	Total nitrogan in 100 gr. dried blood.	NO_3-N_2 accumulated in 100 gr. peat + 20 gr. dried blood.
%	%	%	%
0.8	0.002	11.5	0.014

Ammonium sulphate. Ammonium sulphate has been added to the Park Belt soil to obtain what is called its "nitrifying power", which means the amount of nitrate nitrogen produced per unit weight of soil. The results are quite arbitrary for normal soil conditions are displaced, but the results find their value in a comparative sense with other soils under similar conditions. Under normal circumstances ammonium sulphate is so decomposed that an acid reaction would soon develop if sufficient base forming elements were not present to counter this, and so tend to inhibit the process it was intended to stimulate for nitrification is best carried on in a neutral or slightly alkaline medium. To obviate this calcium carbonate was added.

Table 6. - Comparison of soils under different treatments.

Treatment - Water.

Soil group.	Ratio organic matter to nitrate nitrogen.		Mgs.nitrate nitrogen per 100 gr.soil.	Comparative value.
	Org.matter mgs.	NO_3-N_2mgs. per g. org. matter.		
Prairie	1,724	.58	4.2	77
Park	4.166	.24	6.6	32
Timber	1,333	.75	16.7	100
Peat	40,000	.025	2.3	3

Table 7. - Treatment - Phosphorus.

Soil group.	Ratio organic matter to nitrate nitrogen.		Mgs.nitrate nitrogen per 100 gr.soil.	Comparative value
	Org.matter mgs.	NO_3-N_2mgs. per g. org. matter.		
Prairie	1,613	.62	4.50	100
Park	2,381	.42	11.54	77
Timber	2,381	.42	9.38	77
Peat	40,000	.024	2.21	3

Comparison of soils under different treatments (continued).

Table 8. - Treatment - Calcium Carbonate.

Soil group.	Ratio organic matter to nitrate nitrogen.		Mgs.nitrate nitrogen per 100 gr. soil.	Comparative value.
	Org.matter mgs.	NO_3-N_2 mgs. per g.org. matter.		
Prairie	1,449	.69	5.00	100
Park	2,702	.37	10.00	50
Timber	2,702	.37	8.2	50
Peat	22,222	.045	4.2	7

Table 9. - Treatment - Calcium Carbonate and Blood.

Soil group.	Ratio organic matter to nitrate nitrogen.		Mgs.nitrate nitrogen per 100 gr. soil.	Comparative value.
	Org.matter mgs.	NO_3-N_2 mgs. per g.org. matter.		
Prairie	917	1.09	10.00	20
Park	200	5.00	145.4	100
Timber	526	1.90	45.7	39
Peat	1,282	.78	72.7	16

Comparison of soils under different treatments (continued).

Table 10. - Treatment - Blood.

Soil group.	Ratio organic matter to nitrate nitrogen.		Mgs. nitrate nitrogen per 100 gr. soil.	Comparative value.
	Org. matter mgs.	NO_3-N_2 mgs. per g. org. matter.		
Prairie	378	2.61	24.00	55
Park	218	4.58	133.3	100
Timber	606	1;65	40.0	36
Peat	6,666	.15	13.70	3

Table 11. - Increase in the accumulation of nitrates and numbers of bacteria when ammonium sulphate was added to Park Belt soil.

Period of Incubation in weeks.	Control.		$(NH_4)_2$ SO_4	
	NO_3-N_2 parts per million.	Number millions per g. of soil.	NO_3-N_2 parts per million.	Number millions per g. of soil.
1	20.3	6.1	24.4	7.8
2	50.0	3.0	72.7	7.5
3	60.0	1.5	271.1	1.5
5	46.9	3.0	355.5	3.2
8	69.8	1.5	410.0	0.
11	100.0	10.5	516.0	6.0

It is very obvious from the figures given in Table 11 that the addition of ammonium sulphate stimulated nitrification throughout the whole experimental period. The additive effect of easily assimilable nitrogenous material is apparent too in the initial increase in bacterial numbers; and then the crux of the significance of this treatment is revealed in the range of the particular soil to decomposed nitrogenous material, indicating that, other factors being equal, the balance of groups of the micro-organisms of the Parkland soil is actively maintained and conducive to a fertile environment for plant growth.

In endeavouring to interpret the figures resulting from chemical treatment we cannot assume that any increment or decrease in any factor measured is directly attributable to the added substance, seeing that the addition of a definite quantity to the four soils might be sufficient to cause beneficent results in one, whereas it might depreciate the same factor in another, but, on the other hand a half of the quantity might give effective results.

The effect of the different treatments on nitrification is set forth in Tables 6-11 inclusive, and the biological data are included in the tabulation of the whole of the results as shown in Table 15, p.70.

It is evident from these results that there is no dearth of nitrate nitrogen in our mineral soils when it is considered that approximately 58 pounds of nitrate nitrogen per acre are required to produce a thirty bushel crop of wheat, but a similar condition does not pertain to our peat soils, When a comparison is made of the nitrogen content of the mineral soils and peat, see Table 1,

p. 15. It will be at once obvious, that if only .8 grams of nitrogen is contained in 93.13 grams of organic matter as opposed to .9 grams of nitrogen in 27 grams of organic matter in the Park Belt soil, there is the probability of nitrogen soon becoming, if it is not already so, a limiting factor in crop production. Then again, the fact must not be lost sight of that the nitrogen content of equal volumes of, say, Park Belt soil and peat, is even more divergent than when calculated on a percentage basis. It might be objected that upon the addition of calcium carbonate there was a reasonable amount of nitrates formed but it must be appreciated that the specific gravity of peat would necessitate spreading this over a much wider area than would be covered by an amalagous quantity of mineral soil, and that the rate of nitrate productivity is only one-seventh that of the lowest of our mineral soils in the calcium treated sample, also the addition of calcium carbonate to acid peat causes sooner or later denitrification (51) which is very forcibly demonstrated in this experiment after the first week's incubation.

From this the writer gathers that there is a much smaller proportion of nitrogenous organic matter present per unit of material and that its release is either limited by this fact (its effect upon physiological activity of microorganisms), or if it is released its subsequent formation into nitrate nitrogen is inhibited owing to the peculiar microbial population which assimilates ammoniacal compounds. With this ambiguous condition before us, it was thought desirable to investigate the matter further with a view of endeavouring to correlate another factor along with the nitrate radical so as

to ascertain whether decomposition was active in spite of lack of nitrate production. Seeing that biological activity is one of the primary factors concerned in decomposition, it appeared logical to suppose that a measure of oxidation would reflect a condition of disintegration of organic matter, and with this in view a preliminary survey was made upon the feasibility and significance of measuring the carbon dioxide evolved from the decomposition of peat, which forms the second part of this thesis.

Decomposition of Peat Measured by Carbon Dioxide.

The peat that has been used throughout this part of the experiment was obtained last fall from a drained bog situated along the Stony Plain Road about seven miles west of Edmonton. It is featured by a covering of sphagnum, intermingled with which is Ledum groenlandicum as the dominant shrubby ericaceous plant, and a sparse forest growth of Larix and Picea sp.

In the reclamation of peat bogs drainage would be resorted to as a primary condition, which would mean that in the utility of the peat for agricultural purposes the "subsoil" or the mineral foundation upon which this vegetation is resting must play an important part along with the organic material in creating a suitable environment for microorganic activity, so it was thought desirable to make a profile of a typical section, take samples of each horizon, and analyse them for calcium, carbon, and nitrogen.

It is a well known fact that the organic matter of the mineral soil soon loses its definite plant structure, a reflection of decomposition, but that of peat is definite and discrete having all the earmarks of living vegetation.

Plate III. Photomicrograph of stainless preparation of organic matter from peat. Note definite structure. X1200

Analyses of Peat Profile.

It will be observed from Table 12 that there are three distinctive layers of organic matter, each conforming to a different carbon nitrogen ratio, not in the form of a smooth gradient from the surface to the interior as one might suppose,but rather presenting a condition that suggest we must look at some other factors in juxtaposition to the lowest layer of organic matter for an explanation why such a relatively low carbon nitrogen ratio at the extreme interior of the peat bed can exist. Bearing in mind the fact that we are dealing with a drained bog in which no gravitational water is present in the organic matter, there exists throughout the peat a free interchange of soil and atmospheric gases a condition, although in itself a prime factor of aerobic decomposition, cannot explain the wide carbon nitrogen ratio of the intermediate layer, but observation of the calcium content of the mineral subsoil suggests to the writer that the calcium of the layer adjacent to the lowest peat stratum has played an important part in furthering decomposition of that layer by neutralising the acidic conditions therein and so creating a more favourable environment for biological activity. There is then the rather interesting fact which suggests that decomposition is taking place from opposite directions, a condition substantiated by the carbon nitrogen ratios of the different layers of organic matter. We see here the possible significance of the part that the mineral subsoil may play in a system to create a suitable environment for the growth of higher plants, and the position it must occupy in any consideration involving the fertility of our peat deposits.

Circumstances at the time of sampling were opposed to making a biological study of these different strata.

Table 12. - Analyses of Peat Profile.

Vertical Formation	Depth	Carbon	Nitrogen	C/N	Calcium	Water
	in.	%	%		%	%
Fairly dark colour. Partially decomposed peat.	7	43.4	1.31	1:33	.71	79.11
Light colour; Raw peat.	4	41.1	.95	1:43	1.22	90.96
Darker in colour than top layer.	12	39.1	1.80	1:22	3.20	86.30
Sub-Soil (Gradation from granular structure to firm clay.)						
Considerable dry material admixed.	12	-	0.11	-	.85	25.44
Trace of organic matter.	12	-	0.05	-	2.35	24.63
No organic matter.	12	-	0.02	-	3.25	20.28

Carbon was determined by the Liebig combustion method.

Moisture calculated on wet basis.

Is nitrogen a limiting element?

The results of the experiment regarding the rate of nitrification of peat as well as the limited amount of nitrogen per gram of organic matter present compared to that in the organic of the mineral soils, indicated that nitrogenous substances were not readily available. These conclusions were subsequently confirmed by the results of the carbon nitrogen analyses, which showed an extremely wide C/N ratio compared to mineral soils even where decomposition had been most active in the peat.

From previous studies of the microbial population of peat it was observed that the dominant group was fungi, which, under suitable conditions, are very active in cellulose decomposition, but when the relatively enormous quantity of organic material per unit of peat soil is considered, the microorganic numbers are small. The numbers are limited amongst other things by the unavailability of nitrogen; the addition of this element would stimulate physiological activity, which would be reflected in the decomposition of organic matter, with a consequent release of nitrogenous substances, which would, under suitable conditions, be metabolised into nitrate nitrogen for assimilation by vegetation.

This suggested that it would be necessary to add some form of easily decomposable nitrogenous material and it occurred to the writer that fixation of atmospheric nitrogen might be possible under certain conditions.

Fixation of free atmospheric nitrogen in solution.

Our first concern then was to provide a suitable environment for the culture of free nitrogen fixing organisms

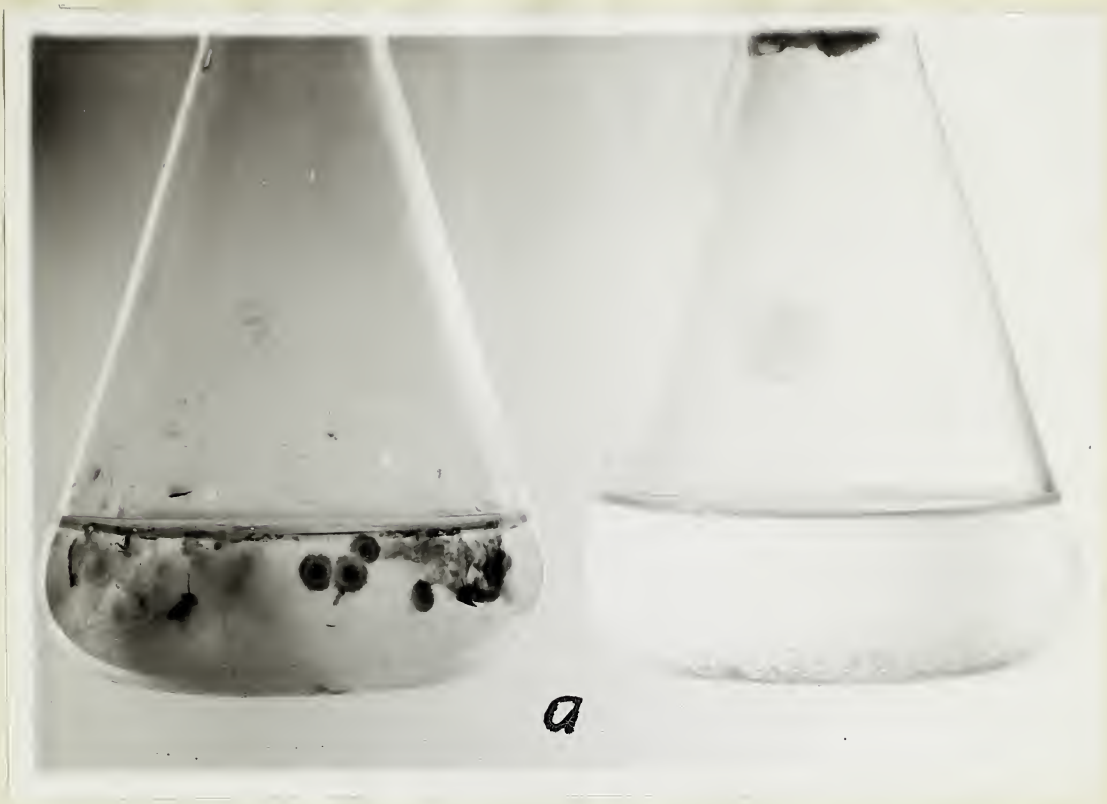
which had previously been isolated from the field immediately north of the Agricultural Building. Ashby (3) found that dextrose provided an excellent source of energy for these bacteria, and as numerous experiments (57) have demonstrated the possibility of obtaining monosaccharides by hydrolysis of cellulose, a series of experiments were carried out with Marquis wheat straw and phosphoric acid,of variable concentrations, in an endeavour to obtain reducing sugars suitable as a source of energy for free nitrogen fixing organisms. Observation of Table 13 shows that there was in increment in reducing sugars, expressed as dextrose, for each increase in the concentration of phosphoric acid.

Table 13. - Reducing sugars in 100 grams Marquis wheat straw.

Phosphoric acid (H_3PO_4)	Grams of reducing sugars per 100 gr. of straw.
%	%
0.00	3.12
0.45	3.60
1.00	4.05
2.00	5.07
3.00	5.28
4.00	5.47
5.00	5.68

Determination by Bertrand method.

Though these results were encouraging it was necessary to test the efficiency of the reducing sugars as a source of energy for the microorganisms by inoculating with the specific bacteria a series of the respective sugar solutions and incubating for 32 days.



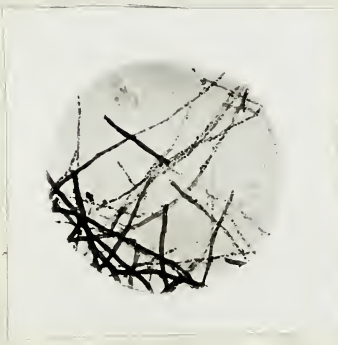


Plate IV. Isolation of atmospheric nitrogen fixing organisms. (a) Isolation of crude cultures in Dextrose-nutrient Salt Solution. The circular colonies shown were taken out of flask and slide (b) Azotobacter made under sterile conditions. (c) A characteristic growth of Azotobacter. b & c photomicrograph magnification X1200.

Three such series in all were carried out, but the result of the first prompted the utilization of the method and the subsequent two series were made as confirmatory tests of the initial one. Reference to Figure 4 (p.47a) indicates that free atmospheric nitrogen fixation was an accomplished fact, and steadily increased with each increment of reducing sugar corresponding to the percentage acid solution. It was not possible to analyse the solutions at the end of the incubation period and to calculate the rates between the sugar - energy - metabolized and nitrogen fixed, but the significance of this biological phenomenon is that given available energy in the form of assimilable carbohydrates and absence of nitrogenous material, free atmospheric nitrogen is fixed as cellular protein.

Evolution of carbon dioxide a measure of decomposition.

Satisfied then that the straw filtrate contained the energy, and nitrogen fixation was an accomplished fact, the filtrate resulting from the 2% acid hydrolysis of straw was introduced into the incubation chamber, and the main experiment commenced of measuring the carbon dioxide evolved daily over a period of two weeks.

In all instances where inorganic salts were added there was a marked increase of carbon dioxide over the control, and in cases where organic substances were added the increases were accentuated. This latter condition is not difficult to understand in the light of the fact that under normal conditions there is an unappreciable amount of easily assimilable food substances for microbial attack, but the addition of sugars furnishes available

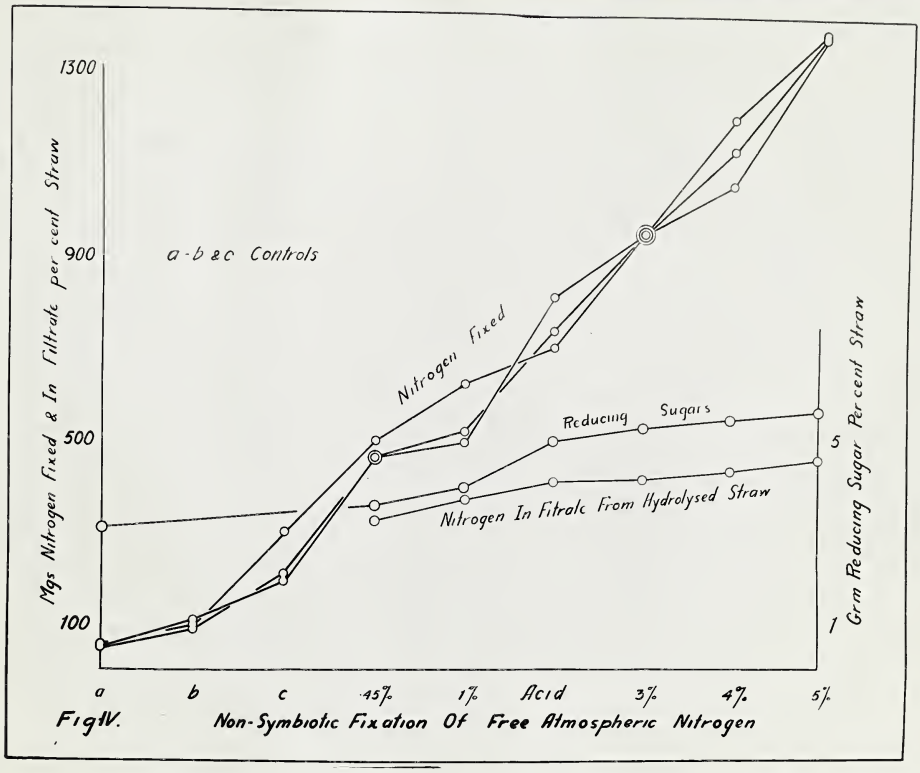

1300
900
500
100
Mgr Nitrogen Fixed & In Filtrate per cent Straw
a-b & c Controls
Nitrogen Fixed
Reducing Sugars
Nitrogen In Filtrate From Hydrolysed Straw
5
1
Grm Reducing Sugar Per cent Straw
a
b
c
·45%
1%
Acid
3%
4%
5%
Fig IV.
Non-Symbiotic Fixation Of Free Atmospheric Nitrogen

Figure 4.

energy, the assimilation of which is reflected in increased amounts of carbon dioxide evolved.

Carbon dioxide and bacterial numbers.

It had been observed in the nitrification experiment that there were conditions peculiar to biological activity. We saw, for instance, that there was a constant rhythm of bacterial numbers, and seeing that this condition reflects the expenditure of energy there should be a fluctuating reflection in carbon dioxide evolution a condition found by Russell and Appleyard (53) which should be relatively constant for the treatment under experiment.

Reference to Figure 5, wherein a graphical representation of carbon dioxide evolved is made of the treatment giving maximum readings as against the control, from which it is apparent that the factor responsible moves in a definite cycle comparable to variations in bacterial numbers.

Granted then that carbon dioxide plays a primary part in soil economics, as an indicator of decomposition of organic matter, making soluble other essential substances as well as augmenting the atmospheric carbon dioxide, and so increasing photosynthesis (37) which, however, is limited by certain external factors ( ), the fact must not be lost sight of that nitrate nitrogen must always be readily available, for any process that is conditioned by several factors the rate of growth is limited by the speed of the lowest one (8).

Neither must we be blind to experimental facts which have shown that certain food substances, for instance carbohydrates and proteins, may be interchangeable for energy purposes, the decompo-

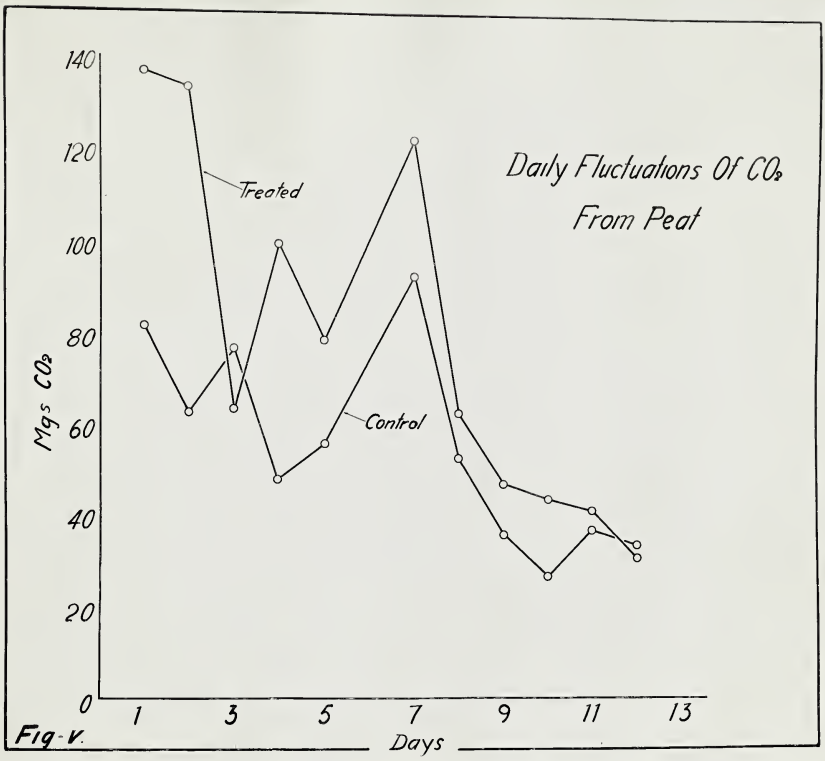

Daily Fluctuations Of CO_2
From Peat
Treated
Control
Mgs CO_2
140
120
100
80
60
40
20
0
1
3
5
7
9
11
13
Days
Fig. V.

Figure 5.

sition of the nitrogenous material being at a minimum in the presence of easily assimilable carbohydrates (32). In these circumstances a high amount of carbon dioxide would be evolved and yet the soil conditions remain infertile.

Carbon dioxide and nitrate nitrogen - Effect of treatment.

Reference to Table 14 shows the relation between carbon dioxide and nitrate nitrogen for the different treatments at the end of fourteen days. In the instance where the uninoculated sample receiving nutrient salts and calcium carbonate resulted in an appreciable increase of nitrate nitrogen, it will be observed that the total carbon dioxide evolved is less than in its inoculated counterpart, which also showed a much smaller accumulation of nitrate nitrogen. This may be accounted for by the fact that the nitrifiers, bacteria responsible for nitrate formation, utilize only the carbon of carbon dioxide for cellular material.

The addition of straw filtrate resulted in a very marked increase in carbon dioxide and nitrate nitrogen over the control, but it must be observed that there is not any appreciable difference between the nitrate nitrogen of this and that where nutrient salts and calcium carbonate were added, which condition emphasizes the fact that the ratio carbon dioxide/nitrate nitrogen may be greatly displaced by the addition of an easily assimilable carbonaceous nutrient. This condition suggests the importance of establishing a narrow carbon nitrogen ratio in peat, and any effort which tends to effect this is a step towards fertility. This fact may not be apparent immediately, for observation of the treatment of ammonium sulphate and calcium carbonate has resulted in no

Table 14. - Peat under different chemical treatments: Carbon Dioxide evolved & Nitrate Nitrogen accummulated in 14 days, from 25 grammes of Peat.

	CONTROL		$CaCO_3$		$CaCO_3$ and Nutrient Salts		Nutrient Salts		$CaCO_3$ & $(NH_4)_2SO_4$		Carbohydrate (Sugars)		Carbohydrate (Starch & Diastase)	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Carbon Dioxide mg.	595.20	703.54	732.27	711.21	662.25	789.94	755.42	778.77	648.63	751.81	1074.98	1034.44	863.97	823.98
Parts per Million NO_3- N_2	36.05	35.40	40.55	39.05	45.75	37.70	33.95	35.20	36.05	31.80	47.45	47.15	33.80	37.90

Odd numbered samples uninoculated.

Even numbered samples inoculated with mixed cultures free nitrogen fixing organisms.

increase in nitrate formation, a result that is traceable to the microbial population peculiar to the peat under investigation, for any nitrate nitrogen formed, as well as the ammonia of the ammonium sulphate, may be utilized by the microorganisms.

Microorganisms of peat and reaction to treatment.

From our previous investigation of the biology of peat the evidence is convincing that the microbial population is wholly different, at least in dominant groups, to that of mineral soils. It will be observed, for instance, that there is present a dominant fungoid population, which reflects a very wide range of adaptability to acidity, but because of the decidedly acid nature of peat it predominates.

Fungi of the soil can assimilate various carbohydrates and most species can obtain their energy from cellulose, in which respect they differ from many bacteria (5), and, on the other hand, they can readily assimilate ammonia and nitrates, but have no power to oxidize ammonia to nitrates (51). It is not difficult to see then why the addition of ammonium sulphate had no effect on nitrate production during the time of the experiment, but it must ultimately tend to narrow the C/N ratio by becoming incorporated as microbial protoplasm.

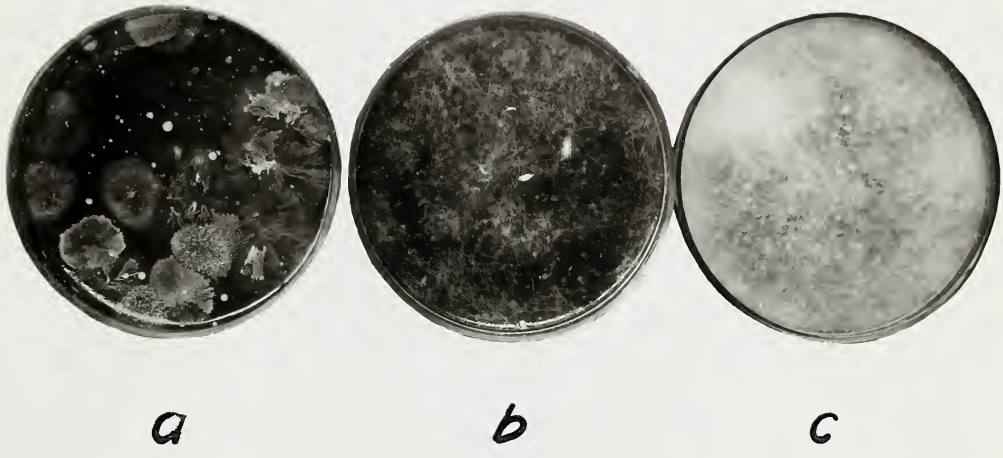


Plate V. Plate Cultures from Peat.

(a) Total bacterial counts.
(b) Actinomyces.
(c) Fungi.

The fixation of free atmospheric nitrogen by mixed cultures of the specific organisms in the filtrate treatment could not be definitely established owing to the short duration of the experiment.

	Control.	Treated sample.
Total nitrogen.	.8%	.87%

but the increase is suggested, although not necessarily significant, owing to the complexity of the organic material with which we are dealing. A qualitative test for nitrogen fixing organisms showed their presence in all the incubated samples excepting the control. There is no question that the addition of the carbohydrate had a beneficial effect seeing that nitrates accumulated, and the rate of carbon dioxide evolution was greatly accelerated.

From our previous discussion it is obvious that in peat soil we have to contend with a microbial population which is antagonistic to soil fertility, and the question naturally arises, has the addition of the various chemical substances had any influence on the dominancy of the microorganic groups? To answer this question all the different treatments were plated out in triplicate on specific media for the isolation of (a) bacteria, (b) fungi, and (c) actinomyces; and, in no instance, was there any change in the numbers or groupings of specific colonies from that of the control; which leads the writer to suggest that although nitrate accumulation was an established fact in one or two cases, it would not necessarily be permanent but rather transitory, owing to the addition of substances which initially displaced that

biological condition which reflects the history of a very stable environment, and that sooner or later competition for nitrate nitrogen would set in between microorganic and plant growth. It is an established fact that nitrates accumulate, and later disappear, when peat is treated with calcium carbonate (51), a condition also confirmed in our own experiment.

Carbon dioxide as a measure of fertility!

In mineral soils the measure of carbon dioxide may be used in grading their fertility (71), and although in peat we are dealing with an abnormal plant environment, it was thought desirable to test out the practicability of the method in respect to the sample which had been modified by the addition of the simple carbohydrate resulting from the treated straw.

Definite quantities of peat (200 grams) were weighed into earthenware crocks, the necessary constituent added, and in each was planted a dozen Victory oat seeds. The crocks were placed in the greenhouse and subjected to controlled conditions as regards temperature, light and water. Germination was even and rapid in each container, but in a very short time a variation became apparent, probably immediately after the seed had become exhausted of food. Nos. 1 and 5 showed very little growth, the culms remained spindly and the colour of the vegetation indicated a lack of nitrogen. The remainder showed progressive growth, but Nos. 3 and 4 gradually exceeded No. 2, and the colour of the formers' vegetation was analogous to vegetation growing in a normal habitat; but it soon became evident that there was some condition interfering with the normal plant metabolism of Nos. 3 and 4, for the tips of the plants began to fade from the apex

down, which condition suggests a lack of potassium, a condition which is prevalent in some of the muskegs of the United States. (17).

It is interesting to note that as a result of our preliminary investigation of the decomposition of peat by the carbon dioxide method, there is indicated an apparent lack of potassium, a condition of which we had previously no evidence.

Plate VI.

No. 1 = Control.

No. 2 = + $CaCO_3$.

No. 3 = Filtrate from hydrolysed straw + $CaCO_3$ + nutrient solution.

No. 4 = " " " " " "

No. 5 = Nutrient Solution.

Photo does not show distinguishing colours of vegetation. Nos. 1-2 & 5 are very light, but Nos. 3 & 4 are quite normal except that numerous leaves have died back from the tips and others are becoming mottled from apex downwards. If this had not occurred vegetation in Nos. 3 & 4 would have been very profuse as compared with the others; the culms are also much thicker and stronger and the leaves broader than No. 2

SUMMARY.

1. Four soil groups of Alberta have been subjected to specific treatments and incubated under laboratory conditions for definite periods of time.

2. A measure of biological activity, i.e., bacterial numbers, ammonification and nitrification, has been made and recorded.

3. Where the optimum quantity of water was added, there was a direct relationship between the organic matter, bacterial numbers and nitrification.

4. A definite inverse relationship is shown between the number of microorganisms per gram of organic matter and the total organic matter in 100 grams of soil.

5. An explanation, involving a fundamental concept in soil bacteriology is suggested to account for the fertility pf semi-arid soil (Prairie).

6. The Soil Groups, Timber, Prairie, Park and Peat, under optimum moisture conditions, are arranged in a definite order of nitrifying power.

7. Results of nitrification in the soil groups under different fertilizer treatments are tabulated and the soils arranged in their order of nitrifying power.

8. Experimental evidence supports the fact that nitrogen is a limiting element in the peats under experiment.

9. The beneficent effect of blood on peat is particularly outstanding.

10. A profile has been made of a peat bog, the surface layer of which has been used in decomposition studies by measuring the carbon dioxide evolved. C/N ratios of three strata of this profile have been made. A preliminary investigation of the possibilities of induced decomposition of peat by fixation of atmospheric nitrogen by biological agency has been attempted; there is no conclusive evidence of nitrogen fixation. Measurements of carbon dioxide evolved from decomposition of peat under different treatments have been made, which indicate enhanced decomposition in certain instances.

11. The microorganisms of peat and mineral soils are shown to be wholly divergent in dominant groups.

12. As a result of the carbon dioxide studies certain of the treatments were utilized and studied in connection with plant growth, which indicated that potassium might be a limiting factor.

CONCLUSIONS.

Organic matter, Microorganisms and Nitrate Nitrogen, are interrelated factors, and are indicative of a condition of fertility. The measurement of the relative rates at which definite quantities of different soils form nitrates in a definite period of time should be based upon the unit of organic matter present. The fertility of soils is explainable on that basis.

Any addition to the soil which displaces this relationship would sooner or later be opposed to fertility, but the divergence of these factors permissible for a fertile soil is dependent upon the rate at which nitrate nitrogen is made available to the growing plant, which is something we do not know.

Experimental evidence suggests a reason for the fertility of our Prairie soils which are so relatively low in organic matter.

The disparity between the organic matter content and the volume of equal quantities of mineral soils and peat, as well as the inferior percentages of nitrogen in the latter, do not permit of any comparisons being made in relation to nitrification; but if comparisons are made on equal organic matter contents, then the inferiority of peat in relation to total nitrogen content as well as rate of nitrification is definitely developed and points to two reasons for infertility.

The constantly increasing numbers of bacteria of the Prairie soil for each period of incubation, as opposed to the condition of constant flux of the others for a greater number of periods, suggest the necessity of frequent bacterial counts.

The value of a narrow C/N ratio is seen in the blood treatment of peat; nitrates accumulated and initial biological groupings were displaced to more nearly coincide with those of a fertile mineral soil.

From these studies, as a generalization, it might be observed that the quantity of organic matter has an important bearing upon its rate of decomposition, and consequently longevity of fertile soil: For instance -

(a) Low amount of organic matter and fertile soil, indicates rapid oxidation of organic matter, large bacterial numbers per gram of organic matter, rapid rate of nitrification per gram of organic matter, and a short period of fertility, for example, Prairie soils. The duration being conditioned primarily by rainfall.

(b) Intermediate amount of organic matter as compared with peat, and fertile soil indicates less rapid oxidation of organic matter, smaller bacterial numbers per gram organic matter, less rapid rate of nitrification per gram organic matter, and longer period of fertility, for example, Park Belt, Timber.

(c) High amount of organic matter and non-fertile soil, indicates low rate of oxidation of organic matter, very small bacterial numbers per gram organic matter, extremely low rate of nitrification, and indefinite period of low fertility; and these three conditions reflect the correlation Organic Matter-Biological Numbers-Nitrification.

The measurement of carbon dioxide alone from raw peat cannot be used as an index of fertility because the dominant

groups of microorganisms present are opposed to that condition.

When carbon dioxide measurements are supplemented by bacterial plate counts, a condition of fertility is indicated.

Increased nitrate nitrogen accumulation and increased carbon dioxide shortly after treatment do not necessarily indicate increased fertility; the microorganic population may be such as to later cause keen competition with higher plants for nitrate nitrogen.

The dominancy of the fungoid population must be displaced by creating conditions which will permit the ascendency of bacterial groups. This may be accomplished by the addition of simple carbohydrates such as dextrose.

The results of narrowing the C/N ratio may be indicated by a comparison between the three factors: (1) bacterial numbers, (2) carbon dioxide, (3) nitrate nitrogen of the Control and treated sample. There is an indication that at least another limiting factor - potassium - is present in peat.

ACKNOWLEDGMENTS.

To apply the fundamentals of scientific agriculture necessitates a knowledge and appreciation of the significance of those phenomena which it is customary to segregate under the caption - Natural Sciences. To those gentlemen of the Departments of Bacteriology, Botany, Chemistry and Geology, of this University, who, through their able interpretation of natural phenomena, have inculcated in me an enthusiasm for a deeper understanding of the maize of biological activity, I desire to take this opportunity to say 'Thank you'.

To Dr. Robert Newton who, by his thorough elaborations of the laws of those closely allied sciences, Botany and Chemistry, presented to me in one of my courses in my senior undergraduate year, oriented scientific agriculture to a plane rightfully its own, I wish to extend these few words of appreciation.

The problem for investigation - The Biological and Chemical Activities of Alberta Soils - was suggested by Dr. F.A. Wyatt, Head of the Department of Soils, to whom many thanks are due for his generous and helpful suggestions, which could only come from one with such a wide experience of the practical significance of soil science.

Grateful acknowledgment is made to Dr. J.D. Newton, under whose direction this investigation was carried out, for his continued interest and constructive criticisms during the carrying out of this research.

Acknowledgment of indebtedness is extended to Dr. R. Newton, Head of the Plant Biochemistry Department, for his

courtesy and help in permitting the use of certain apparatus and for photographic facilities; to Mr. Cyril Kenway, Field Crops Department, for taking the photographs used throughout this thesis; to Mr. S. Ward, Soils Department, for his assistance in making the profile of the stratification of the Peat Bog under experiment in this investigation; and to the Dominion Government, Department of the Interior, Natural Resources Branch, for copies of the frontis map.

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Table 15. - Complete details of Nitrates, Ammonia, & Micro-organisms of Four Soil Groups investigated.

TREATMENT.		WATER.				PHOSPHOROUS				CALCIUM CARBONATE				CALCIUM CARBONATE & BLOOD MEAL				BLOOD MEAL				AMMONIUM SULPHATE.
Period of Incubation.	Product Measured.	Park-land.	Prairie.	Timber.	Peat.	Park-land.	Prairie.	Timber.	Peat	Park-land.	Prairie.	Timber.	Peat.	Park-land.	Prairie.	Timber.	Peat	Park-land.	Prairie.	Timber.	Peat.	Park-land.
One Week.	NH_3- N_2	11.72		12.84	26.74	9.30		14.94	10.82	21.59		17.96	15.48	112.50		109.20	216.80	106.85		84.54	114.90	56.95
	NO_3- N_2	1.09		1.56	8.21	1.67		1.60	3.84	2.03		2.21	6.94	2.65		1.55	3.65	4.88		.80	1.39	2.44
	Bacteria	3.34		4.89	1.26	5.60		3.68	1.39	6.15		4.64	6.27	20.00		34.40	62.40	33.60		56.00	4.51	7.80
	Fungi	-		-	-	-		-	-	-		-	-	-		-	-	-		-	-	-
	Actinomyces	-		-	-	-		-	-	-		-	-	-		-	-	-		-	-	-
Two Weeks.	NH_3- N_2	7.74	4.48	15.54	49.38	10.19	4.48	13.20	16.58	7.65	6.34	11.94	14.34	116.80	99.60	64.17	172.40	110.10	101.40	80.50	207.60	40.99
	NO_3- N_2	2.03	1.47	5.88	6.94	3.19	.93	5.00	1.85	5.00	1.70	4.69	5.10	7.84	.37	8.89	6.88	1.61	.38	8.94	1.63	7.27
	Bacteria	1.60	2.60	1.65	1.60	3.20	1.30	9.60	1.50	3.00	1.30	4.80	6.00	22.40	14.30	51.20	182.00	19.20	22.10	19.20	150.00	7.50
	Fungi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Actinomyces	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Three Weeks.	NH_3- N_2	9.95		7.14	26.70	7.60		4.82	14.36	9.61		4.49	42.22	116.20		54.42	336.00	135.30		61.35	497.60	39.52
	NO_3- N_2	2.78		5.88	2.43	5.00		7.14	2.00	6.00		6.52	5.05	30.18		14.81	4.90	24.24		13.44	1.55	27.12
	Bacteria	1.60		1.61	1.57	1.60		1.60	1.40	1.50		1.60	13.50	12.80		11.20	352.00	14.40		4.80	300.00	1.50
	Fungi	.11		.11	.19	-		-	-	-		-	-	-		-	-	-		-	-	-
	Actinomyces	.29		.28	.09	-		-	-	-		-	-	-		-	-	-		-	-	-
Five Weeks.	NH_3- N_2	11.75	9.32	8.35	93.22	9.30	8.14	7.10	87.08	12.44	5.76	7.48	22.66	73.82	115.20	60.10	478.00	80.66	120.10	53.61	477.60	31.70
	NO_3- N_2	3.57	3.00	8.33	2.48	7.50	2.63	7.14	1.95	4.69	4.39	6.67	6.50	106.66	2.96	19.51	10.20	72.72	4.17	17.58	2.72	35.55
	Bacteria	3.22	5.20	4.95	1.57	3.20	6.50	8.00	1.70	3.00	3.90	3.20	12.80	9.60	9.10	1.60	84.00	12.80	42.90	4.80	too numerous.	3.20
	Fungi	.07	.05	.12	.15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Actinomyces	.58	.45	.69	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Eight Weeks.	NH_3- N_2	6.99		6.64	76.88	6.88		10.47	66.78	6.50		8.28	29.62	49.13		17.08	264.80	90.28		19.39	299.40	18.55
	NO_3- N_2	5.77		13.33	2.29	8.33		10.71	1.66	6.98		8.33	5.00	68.66		23.53	20.83	100.00		28.57	13.33	41.02
	Bacteria	1.57		1.61	3.12	1.60		0.0	1.50	1.50		20.80	1.50	0.0		1.60	12.00	1.60		1.60	4.80	0.0
	Fungi	.28		.39	.14	-		-	-	-		-	-	-		-	-	-		-	-	-
	Actinomyces	.15		.14	.13	-		-	-	-		-	-	-		-	-	-		-	-	-
Eleven Weeks.	NH_3- N_2	9.22	6.19	9.11	112.72	7.26	5.74	7.97	24.92	8.06	6.78	5.64	16.30	44.78	67.62	16.59	355.40	75.34	117.20	20.85	269.60	14.58
	NO_3- N_2	6.62	4.17	16.67	2.27	11.54	4.55	9.38	2.21	10.00	5.00	8.22	4.17	145.45	10.06	45.71	72.72	133.33	24.00	40.00	13.70	51.61
	Bacteria	11.10	7.68	3.28	1.67	4.80	7.91	1.70	1.70	10.50	5.20	1.60	6.00	19.20	11.70	3.20	18.00	9.60	13.00	1.70	50.40	6.00
	Fungi	.33	.11	.50	.37	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Actinomyces	.46	.92	.52	.13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

	$NH_3 - N_2$	9.22	6.19	9.11	112.72	7.26	5.74	7.97	24.92	8.06	6.78	5.64	16.30	44.78	67.62	16.59	355.40	75.34	117.20	20.85	269.60	14.58
	$NO_3 - N_2$	6.62	4.17	16.67	2.27	11.54	4.55	9.38	2.21	10.00	5.00	8.22	4.17	145.45	10.06	45.71	72.72	133.33	24.00	40.00	13.70	51.61
Eleven Weeks.	Bacteria	11.10	7.68	3.28	1.67	4.80	7.91	1.70	1.70	10.50	5.20	1.60	6.00	19.20	11.70	3.20	18.00	9.60	13.00	1.70	50.40	6.00
	Fungi	.33	.11	.50	.37	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Actinomyces	.46	.92	.52	.13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Unheated	$NH_3 - N_2$	.16	3.73	2.94	14.74
Control	$NO_3 - N_2$	.50	.22	.26	1.48
	Bacteria	2.16	.10	1.36	.03
Heated	$NH_3 - N_2$	8.33	6.16	4.74	4.70
Control	$NO_3 - N_2$	.27	.23	.35	1.76

Ammoniacal Nitrogen expressed as parts per hundred thousand

Nitrate Nitrogen " " " " " "

Micro-organisms " " Millions per gramme of soil.